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Mountains are main drivers of speciation and elevational patterns of species richness in New World pitvipers (Viperidae: Crotalinae)

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Abstract. New World pitvipers (*Agkistrodon, Atropoides, Bothrocophias, Bothriechis, Bothrops, Cerrophidion, Crotalus, Lachesis, Metlapilcoatlus, Mixcoatlus, Ophryacus, Porthidium* and *Sistrurus*) are abundantly distributed in the tropics, where they live from sea level to 4,500 m altitude. I analysed the role of mountains in the diversification of the New World pitvipers by quantifying the orographic patterns of speciation and testing the effect of diversification rates and historical colonizations on elevational pattern of species richness. Using a time-calibrated phylogeny I found 42 speciation events, 68.6% of them involving mountain ranges mostly occurring in North and South America. Speciation restricted to lowlands was less frequent and in 75% of the cases happened by separation of an ancestral species by the emergence of mountain barriers. New World pitvipers underwent diversification from an ancestor living at middle elevation, posteriorly leading to several independent colonizations of high and low altitudes. By combining evidence from several methods, I found high initial speciation rates at medium elevations followed by a progressive slowing down negatively correlated with altitude. The effect of the historical processes seems to be less important and only the number of colonizations was positively associated with species richness when 200 m bands, which were correlated with elevation, and 500 m bands were analysed. Thus, evolutionary and historical processes produced an elevational pattern of species richness characterised by a maximum at medium elevations and a sharp decline at the higher.

Key words. Squamata, Serpentes biogeography, macroevolution, molecular phylogeny, speciation.

Introduction

Tropical mountains are pulsars of speciation producing in several groups of organisms high levels of species richness in comparison with neighbouring lowlands (Körner 2004, Antonelli et al. 2009, García-Rodríguez et al. 2021). The causes of why mountains in the equatorial zone have kept this formidable amount of biological diversity is a current topic in macroevolution research (see for example Rahbek et al. 2019a, b). Some researchers have unveiled a common pattern of high species richness at medium elevations with respect to lowlands and the highest elevations of mountains in several groups of vertebrates (Smith et al. 2007, Hutter et al. 2013), leading to the formulation of two non-exclusive hypotheses. The mountain museum hypothesis underlies the historical effects on species richness by proposing that medium altitudes were first and/or more frequently colonized, thus providing more time for speciation (Li et al. 2009, Smith et al. 2007). By contrast, the montane species pump hypothesis emphasized the role of mountains by boosting high diversification rates (speciation minus extinction rates) in medium altitudes (Smith et al. 2007). In tropical mountains, climate experiences a sharp elevational zonation likely playing a pivotal role on the distribution of ectothermal organisms because of their thermal tolerances, especially when dispersal ability of the species is low (McCain 2009, Zuloaga & Kerr 2016, Polato et al. 2018). Patterns of altitudinal species richness in reptiles have attracted less attention, but because of its thermal dependence, it is expected that they experience a decline with the increase of elevation (Jins et al. 2020), although more complex patterns were recovered (McCain 2010).

Vipers (Viperidae) have a wide-world distribution, excluding Antarctica, Australia, Madagascar and Pacific islands, roughly comprising 380 species (Uetz et al. 2023), among which 44% of them are found in the western hemisphere. Viviparity is the most common reproductive mode in vipers (Fenwick et al. 2012) and has allowed them to colonize mesic or even cold areas, reaching 68° N and 47° S latitudes and altitudes of 4,800 m, persisting and even flourishing through the climatic cooling during the Pleistocene (Lynch 2009). The New World viperids are a monophyletic lineage – the pit vipers, subfamily Crotalinae – with strongly phylogenetically supported groups which has more species of vipers than other realms of the earth (Rodriguez et al. 2016) and are widespread along wide altitudinal and latitudinal ranges, including many montane endemics (e.g. *Cerrophidion*, *Mixcoatlus*). Therefore, pit vipers are a suitable model to analyze altitudinal aspects (Jadin et al. 2019) of ecological and evolutionary patterns such as species richness.

Herein, I study how mountains have shaped the diversification of pit vipers in the western hemisphere by examining the congruence between the elevational diversification process through the lineage evolution and altitudinal species ranges. The goal of this paper is (i) to determine the role of the orography in speciation, (ii) to analyse the elevational pattern of species richness and (iii) to test whether high diversification rates and/or older colonizations explain the observed pattern.

Material and methods

Phylogenetic, biogeographic and diversification inferences

I assembled a dataset of six mitochondrial and one nuclear gene fragments of a total 3,965 nucleotides, ranging from 365 to 1,270 bp by gene, of 171 New World pitvipers of the genera *Agkistrodon, Atropoides, Bothrocophias, Bothriechis, Bothrops, Cerrophidion, Crotalus, Lachesis, Metlapilcoatlus, Mixcoatlus, Ophryacus, Porthidium* and *Sistrurus* plus seven viperid outgroups (Supplementary material 1) and visually aligned them using Bioedit 7.2 (Hall 1999). A phylogenetic analysis was performed with previously selected models fixed for each gene using jModelTest 2.1.10 (Darriba et al. 2012) and performing Bayesian inference implemented in BEAST 2.6.7 (Bouckaert et al. 2014) on the most similar models available. BEAST analysis was done using a starting tree based on a Yule model and a lognormal relaxed molecular clock under seven points of calibration well-founded on biogeography and the fossil record (Supplementary material 1). I ran three Monte Carlo Markov chains (MCMC) chains of 10^8 iterations discarding the initial 90%, sampling each 10^4 and assessing for convergence and independent sampling (effective sampling sizes larger than 200) by means of TRACER 1.7.1 (Rambaut et al. 2018). Maximum credibility tree (mc tree) was computed using TreeAnotator 2.6.2 (Drummond & Rambaut 2007) using the 100 last trees of each run. After deleting insular endemics and retaining taxonomically relevant subspecies (see Supplementary material 1 for justification), I used the mc tree of 140 taxa to perform the analysis that comprised the 92% of the non-insular New World endemic viper species in the ReptileDatabase (Uetz et al. 2023).

In order to determine the role of orography on speciation, I performed biogeographic reconstruction of ancestral areas to infer patterns of speciation involving mountain ranges, lowlands or both. Thus, I compiled localities for each species from different sources (Supplementary material 2) in order to estimate its geographic range by constructing digital maps and checked them with IUCN range maps and those provided by diverse bibliographic sources (Campbell & Lamar 2004, Nogueira et al. 2019). Using this information, I assigned each species to primary biogeographic areas (Supplementary material 3) that represent orographic units and plains biogeographically and climatically significant, mostly previously used in biogeography (Bryson et al. 2011, Rovito et al. 2015, Blair & Sánchez-Ramírez 2018, Hamdam et al. 2020). In order to reduce the number of primary biogeographic areas in the analysis and taking into account that the vast majority of the species are restricted to a region of the American continent, I considered the Isthmus of Tehuantepec and the Panamanian-Colombian border as the limits north and south of Middle America and performed separate analysis for North, Middle and South America. A few species had a range that encompassed more than one primary area or regions of the continent. In these cases, I coded species combining a maximum of three areas and choose a combination of the two geographically more distant.

I reconstructed ancestral areas using BioGeoBEARS implemented in RASP 4.2 (Yu et al. 2015) and the mc tree. The models analysed were the dispersal-extinction cladogenesis (DEC), the likelihood implementation of dispersal-vicariance analysis (DIVA) and the likelihood version of the range evolution model (BayArea). I evaluated the fit of the models using the Akaike information criteria (Akaike 1974) comparing their relative corrected (AICc) weights to choose the best model. I aimed to classify the patterns of orogeny leading to two sister species or one species and its sister species pair, to establish categories of divergence by isolation between mountain ranges, within a single mountain range, between a mountain range and its surrounding lowland, within a single lowland area and between two separated lowlands. I discarded ancestral nodes supported by posterior probabilities < 0.90 in the mc tree and those that had the most probably speciation pattern with a probability < 0.75 considering that ancestral area reconstructions failed to likely capture the biogeographic process of speciation. Thus, I summated the probabilities of the ancestral area reconstructions that belong to each previously defined category and chose the orogenic pattern with the highest total probability. I investigated whether the diversification and speciation rates changed through the evolutionary history of New World pitvipers. The accuracy of the available methods to reconstruct diversification rates is a matter of controversy and debate (e.g. Maliet et al. 2019). For this reason, I chose several approaches to compare results and try to achieve independent evidence revealing a common pattern of evolution of diversification rates. Thus, I used Bayesian analysis of macroevolutionary mixtures (BAMM, Rabosky 2014) that explores the variety of potential changes of diversification rates on the phylogenetic tree using reversible jump MCMC and performs a Bayesian estimation of the rates. Priors were estimated using R package BammTools (Rabosky et al. 2014) by means of the function setBammPriors. I ran four chains of 2.5×10^8 iterations, sampling them each 10^5 times, burning out the initial 50% and checked for convergence and enough effective sample sizes using BammTools. I summarised phenotypic rates along the mc tree using the mean phylorate plot depicting the mean phenotypic rate sampled during the iterations and examined the dynamics of diversification rates through time. For comparison, I also used a modification of the birth-death process that allows shifts on the diversification rates at the speciation events and Bayesian estimation of speciation rates using MCMC (Maliet et al. 2019) implemented in Rpanda (Morlon et al. 2015). I ran three chains of 2000 iterations, sampling them each 50, using the model ClaDS2 that accounts for a constant extinction/speciation rate and plot the mean estimated speciation rate for each branch of the mc tree in order to identify differences among lineages. I also examined the variation of the rates through time by performing ordinary-least squared (OLS) regression of the estimated rate of the branches on their half ages calculated as the difference between its two nodes.

Analysis of elevational species richness

I compiled bibliographic data on the maximum and minimum altitudes of the pitviper species, computing mid-elevation points (Supplementary material 3), and classified each one in elevation bands under three different schemes, 200, 500 and 1,000 m, to assess whether results are insensitive to scale of resolution. I compiled bibliographic data on the maximum and minimum altitudes of the pitviper species, computing mid-elevation points (Supplementary material 3), and assigned them to one elevation band to examine the elevation patterns of species richness following the methodology used in other studies (Hutter et al. 2013, Smith et al. 2007). Thus, I performed ordinary least squares (OLS) regression using as a predictor the log-transformed mid-elevation point of the band and the number of species whose elevational range encompassed this band as a response variable. In order to assess whether results are insensitive to scale of resolution, I repeated the analysis, choosing randomly three schemes of band width, specifically 200, 500 and 1,000 m.

To reconstruct the elevational diversification of the ranges, I analysed the species mid-elevational point by modelling its evolution using the available models in R Geiger (Pennell et al. 2014) after testing for significant phylogenetic signal by means lambda statistic implemented in R Phytools (Revell 2012). I transformed branch lengths of the mc tree based on the best model according to the largest AICc relative weight to reconstruct the evolution of the mid-elevation point using the function fastAnc of the R Phytools. I also analysed whether phenotypic rates of mid-elevational point evolution have changed during the evolutionary history of New World pitvipers using BAMM following the same procedure as in the diversification analysis for prior estimation and results analysis, but in this case I ran four chains of 10^9 iterations, sampling them each 10^5 times and burning out the initial 95%.

Testing historical against diversification rate hypotheses

I analysed whether speciation rates are dependent on altitude or historical process of colonization of the elevational zonation can explain differences on species richness. For the first analysis, I used several approaches to overcome the limitations of using one single method and thus checking for the congruent results. Thus, I used the DR species-level metrics of lineage diversification of Jetz et al. (2012) using the command DRstat(tree) implemented in R epm 1.1.1 (Title et al. 2022) that in reality is a measurement of speciation rates in the tips of the phylogeny and therefore is only comparable with true diversification rates when extinction is very low. DR counts the number of splits experienced by the path connecting each species to the root in the dated phylogeny that represent accumulated speciation events, and quantifies the length among splits which is equivalent to the time between these events. Thus, DR uses node density and the inverse of the sums of internode lengths heavily weighing the most recent speciation. To analyse the effect of the altitude on diversification rates, I performed PGLS regression (Orne et al. 2018) of log-transformed elevation mid-point as a predictor on the DR metric of the species using the mc tree. For comparison purposes, I used quantitative state speciation and extinction analysis (QuaSSE, FitzJohn 2010) implemented in R Diversitree (FitzJohn 2012) to investigate the effects of the altitude (mid-elevation point) on the speciation rates. I computed a general mean standard deviation of 377.4 from the species data and a sampling fraction (0.92) estimated using the non-insular species of New World pitvipers listed in ReptileDatabase (Uetz et al. 2023). QuaSSE uses a Brownian motion to model the evolution of a quantitative trait using a birth-death process dependent of the trait, implementing constant (no effect), linear, sigmoid and hump-shaped models. I used the AICc weights to identify the best fitted of the four models accounting for the effect of elevation on speciation rates. However, to address criticism of QuaSEE with regard to problems of phylogenetic pseudoreplication and false discovery rates (Beaulie & O'Meara 2016), I also used the ES-sim test (Harvey & Rabosky 2017) by means of the R implementation essim.R to evaluate whether speciation rates are significantly associated with elevation.

In order to test for historical effects on elevational species richness, I quantified the number of colonizations inferred by the reconstructed elevational mid-points of the altitudinal bands. I assumed that colonizations of non-contiguous bands implied the dispersal through intermediated bands that therefore were also counted as colonizations. Because band colonizations occurred in a lapse of time comprised between the stem and crown ages, to date these events I arbitrarily used the half age between the corresponding nodes. Thus, I performed OLS regressions of the number of colonizations of each elevational band, the age of the oldest colonization and the sum of the age of all the colonizations as predictors on the species richness as a

Table 1. Unambiguously inferred speciation events classified in orographic patterns by continental regions based on well supported nodes and high probable ancestral reconstructions of biogeographic areas.

	Within mountains	Between mountains	Within lowlands	Between lowlands	Between mountains and lowlands	
North America	8	5	4	1	1	19
Middle America	3	4	0	1	0	8
South America	4	2	0	3	9	18
Combined regions	0	3	0	0	0	3
	15	14	10	5	4	48

response variable using log-transformed variables. When regression analysis was significant, I also tested whether the number of colonizations, the age of the oldest colonization and the total time of colonization of the altitudinal band were related to altitude by using the mid-elevation point of the colonized bands.

Results

Biogeographic analysis inferred 62 speciation events that can be assigned to one of the orographic patterns of speciation (Supplementary material 4). After retention of those showing high probabilities of ancestral area reconstruction and their placement in well supported nodes of the phylogeny, the final sample includes 42 events (Fig. 1, Table 1), 60.3% involving sister lineages living in mountain ranges (speciation within mountains or between them) and speciation between mountains and lowlands only in the 8.3%. In contrast, I found speciation related to isolation between lowlands or within them represents 31.4% of the total events. In one pair of *Cerrophidion* and three *Metlapilcoatlus* species, speciation seems to occur by divergence of an ancestor, giving place to descendent species that live in mountains each one on one side of the Isthmus of Tehuantepec, but the vast majority of the events occurred within one the three main geographic divisions of the Americas. Speciation in North American and South American regions accounted for most of the orographic events (39.5%, 37.5%) whereas the contribution of Middle America was only 16.6%, showing different patterns among them. For example, speciation between mountains and lowlands occurred mostly in South America in contrast with speciation within mountain ranges which was more prevalent in North America. For the whole continent, speciation events leading to sister montane species occurred in 48.3% of the cases by isolation in different mountain ranges separated by plains and in 51.7% within the same mountain area. In contrast, in plains 75.0% of the pairs of sister lineages arose by isolation of their ancestor in different lowland areas separated by montane ranges and only in 25% of cases by other barriers.

BAMM analysis revealed initial high diversification rates followed by a progressive slowdown through time (Fig. 2). However, this trend was uniform among lineages, thus rejecting the existence of marked specific rate shifts along the evolutionary history of pitvipers. Inspection of the speciation rates estimated by the birth-death ClaDS2 model with constant turnover (Fig. 2) revealed very small differences among branches in speciation rates and significant decline through time (OLS regression, slope = -1.585 ± 0.569, $F_{1\ 276}$ = 7.751, P = 0.0057).

New World pitvipers have an enormous elevational range from the sea level to 4,572 m in *Crotalus triseriatus* and consequently the calculated mid-elevational point was very wide, ranging among species from 5 to 3,586 m. Species richness tends to decrease with elevation after reaching a peak at 1,000–1,500 m, as evidenced by the negative slope of OLS regressions under the three sampling schemes (Fig. 3). I found significant phylogenetic signal of mid-elevation point (lambda = 0.353, P < 0.0001) indicating that the altitude of the species range was phylogenetically conserved. The lambda model performs better than the others examined by Gieger (relative AICc weight = 0.991, Supplementary material 4) and was used to reconstruct the evolution of elevational midpoint. The ancestor of New World pitvipers was reconstructed as a medium elevation species (Fig. 4a, estimation based on non-transformed values, 1,385 m (95% interval of confidence, 783–1,986 m)) and posteriorly several lineages have independently colonized both lower and higher elevations many times. Rates of elevational evolution were mostly low and uniform through time (Fig. 4b) with the exception of some progressive and recent increases, mostly involving a few low altitude species. Speciation rates were associated with species elevational mid-points, as the three methods used indicated. PGLS regression using the DR metric showed negative relationships between altitude and estimated speciation rates (slope = -0.454 ± 0.196, $F_{1\ 138}$ = 5.357, P = 0.022). QuaSSE discarded the constant and therefore non-effect model (Akaike relative weight < 0.0001), but in contrast the best selected model was not lineal, but hump-shaped with drift (Akaike relative weight = 0.886, Table 2). However, and congruently with the PGLS regression results, ES-sim test performed using 1,000 simulations of the trait under Brownian motion also revealed a negative dependence of speciation on elevation (R Pearson = -0.273, P = 0.035).

Historical impact of band colonization on species richness was only significant in the case of the OLS regressions performed using 200 m and 500 m bands indicating positive association between species richness and the inferred number of colonizations (Table 3), whereas the division in a low number of bands when the 1,000 m categorisation scheme was used precludes the finding of any relationships. However, lower altitude bands were significantly more colonized than those comprising medium or high elevations (OLS regression slope = -0.366 ± 0.149, $F_{1\ 12}$ = 6.018, P = 0.030) for 200 m bands while remaining not significant when 500 m bands were analysed (OLS regression slope = -0.500 ± 0.354, $F_{1\ 5}$ = 1.988, P = 0.217).

Discussion

Mountains played an important role on the massive diversification of New World pitvipers by generating multiple speciation events, producing high endemicity and marked elevational patterns of species richness, as found in other ectotherms such as lungless salamanders (Wiens et al. 2007, Rovito et al. 2015). Mountain uplift during the orogeny of the continent give place to a diversity of climatic barriers mainly isolating mountain ancestors in different ranges separated by plains, barriers within mountains or between lowland ancestors. In contrast only the 20% of total speciation events correspond with isolation

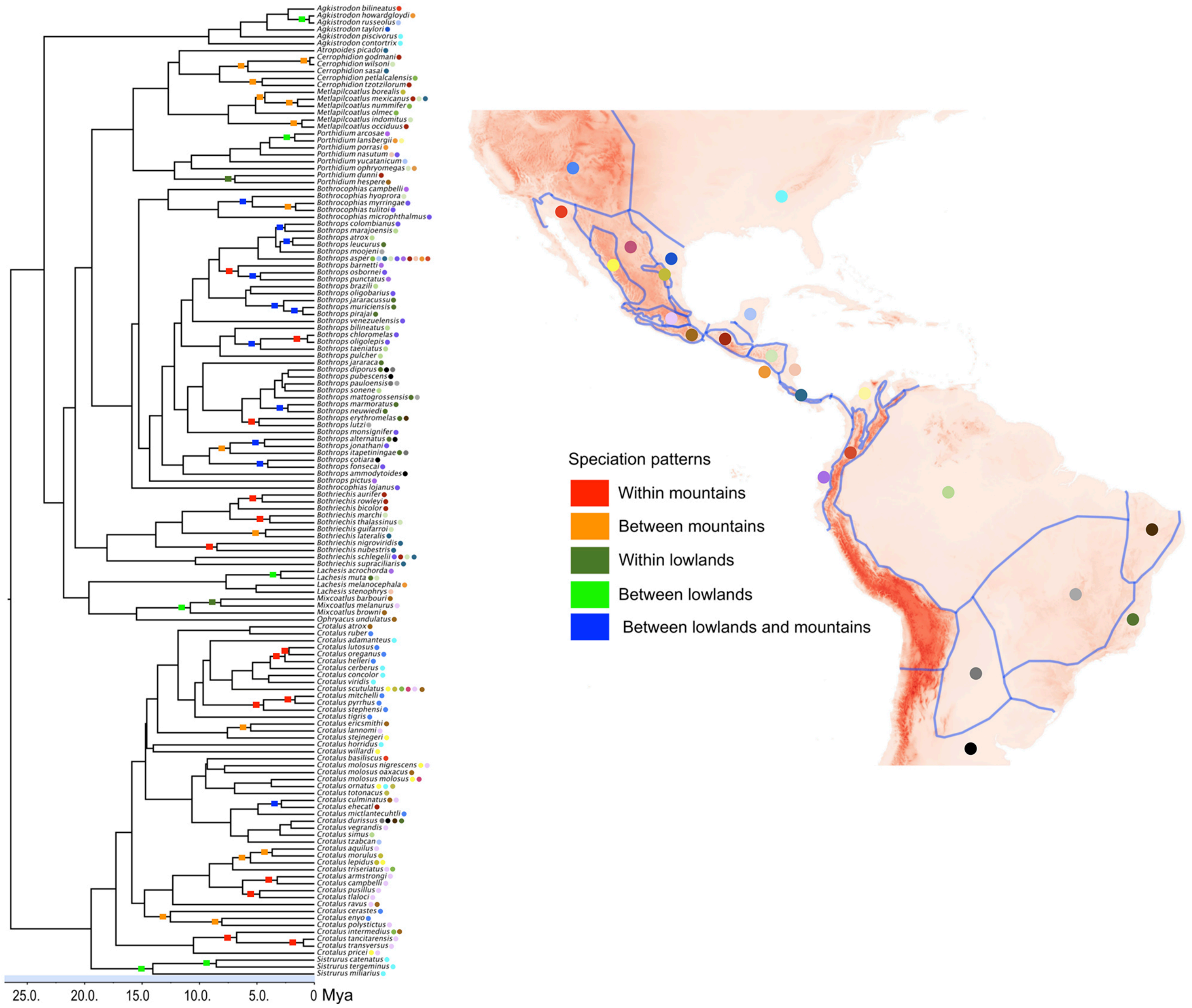

Figure 1. Inferred mode of speciation in New World pitvipers based on ancestral area reconstruction for 42 species groups marked with rectangles on the node: red, both species occur in the same mountain area (speciation within mountains); orange, each species occur in different mountain areas (speciation between mountains); light green, each species occur in different lowland areas (speciation between lowlands); dark green, both species occur in the same lowland area (speciation within lowlands) and blue, one species occurs in mountain areas and the other in lowland (speciation between lowlands and mountains). Biogeographic areas are marked in coloured dots placed in the map and in the phylogenetic tips of each species to represents its geographic range (see Supplementary material 2).

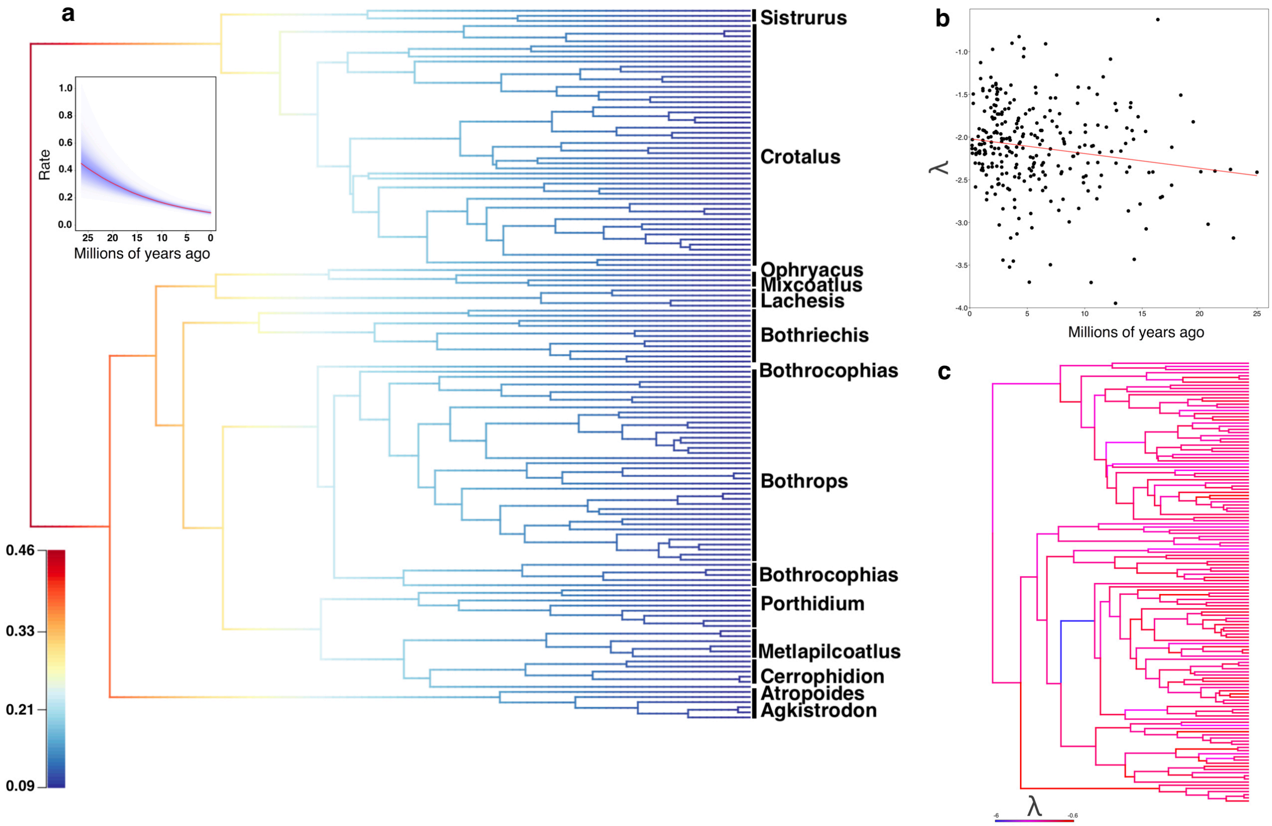

Figure 2. Diversification rate patterns of mainland New World pitvipers: (a) Best scenario according to BAMM showing gradual decreases in diversification rates uniformly along the phylogenetic tree without shifts in groups of lineages. (b) Relationships between speciation rates estimated from the CladeDS and mid-age of the tree branches. (c) Speciation rates heterogeneity in the mc tree from the posterior distribution obtained from CladeDS analysis under the birth-death model with constant species turnover.

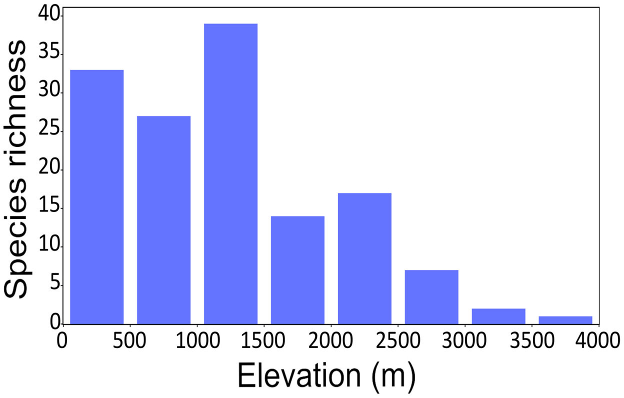

Figure 3. Frequency of species richness by band of 500 m of altitude. OLS regression of the log- transformed variables showed a negative allometry of species richness (slope = -0.503 ± 0.181, $F_{1\,6}$ = 7.717, P = 0.032, adjusted R^2 = 0.489) as well for bands of 200 m (slope = -0.763 ± 0.142, $F_{1\,17}$ = 28.710, P = 0.0001, adjusted R^2 = 0.606) and 1,000 m (slope = -0.020 ± 0.003, $F_{1\,2}$ = 42.550, P = 0.022, Adjusted R^2 = 0.932). Species richness peaks at 1,000–1,500 m (500 m bands), 1,200–1,400 m (200 m bands) and 0–1,000 m (1,000 m bands).

Table 2. Quantitative state speciation and extinction results for elevation dependence on speciation. The best model was hump-shaped with directional drift function.

Model	AICc	Relative AICc weight
Constant	3017.428	< 0.0001
Lineal without drift	3019.414	< 0.0001
Sigmoidal without drift	3020.120	< 0.0001
Hump-shaped without drift	3020.862	< 0.0001
Lineal with drift	2995.392	0.035
Sigmoidal with drift	2993.796	0.078
Hump-shaped with drift	2988.938	0.886

among plains by other barriers than mountains. The active orogeny of the New World continent produced complex mountain systems providing a large amount of opportunities for speciation notably in the Tropical region. For example Andean uplift occurred from 10 to 2 Mya likely separated the common ancestor of *Porthidium ar-*

Table 3. OLS regressions of predictor variables involved in the process of band colonization (number of colonisations, age of the oldest colonization and sum of the ages of the colonisations) on species richness shown for the three schemes of band sampling.

Elevational band		Number of colonizations	Age of the oldest colonization	Sum of the colonization ages‘
	Slope	0.224 ± 0.091	0.097 ± -0.774	0.579 ± 0.260
200 m	P	0.034	0.456	0.500
	Adjusted R squared	0.312	-0.0377	0.264
	Slope	0.137 ± 0.050	0.127 ± 0.088	0.481 ± 0.364
500 m	P	0.042	0.211	0.255
	Adjusted R squared	0.511	0.148	0.098
	Slope	0.026 ± 0.057	0.020 ± 0.079	0.083 ± 0.359
1000 m	P	0.686	0.821	0.837
	Adjusted R squared	-0.352	-0.451	-0.460

cosae and *P. lansbergii* or *Lachesis muta* and *L. acrochorda* but also promoted the expansion of the open/dry diagonal conformed by the Caatinga, Cerrado and Chaco, separating Amazonia from Atlantic Forest (Hoorn et al. 2010). Some vicariant events like the split of the common ancestor of *Bothrops atrox* and *B. leucrurus* or *B. muriciensis* and *B. pirajai* are consistent with the rise of this barrier. If New World vipers colonized lowlands from medium elevation ancestors, as indicated by ancestral reconstruction results, it should be by niche divergence (Schluter 2009) of montane ancestors splitting into sister species retaining the ancestral niche (niche conservatism, Wiens 2004) and others adapted to lowland climate conditions. This orographic pattern of speciation was observed in a few sister species pairs mostly placed in the tropical region, thus validating this assumption. Tropical plethodontid salamanders (Wiens et al. 2007) and glass frogs (Hutter et al. 2013), that share their habitats with pitvipers in the Neotropics also diversified by multiple colonization of lowlands and high altitudes from mid-elevation ancestors.

Despite the potential effects of the glacial oscillations on the species ranges of isolating populations, most of the speciation in New World pitvipers happened before the Pleistocene congruently with that found by previous researchers (Bryson et al. 2008, 2011, Castoe et al. 2009, Mason et al. 2018). These old divergences have proven to be congruent with the formation of three main tectonic events in Mesoamerican *Bothriechis*, *Metlapilcoatlus* and *Mixcoatlus* pitivipers, while in the case of *Cerrophidion* species they are more recent, although in most cases predating the onset of Pleistocene glaciations (Castoe et al. 2009, Daza et al. 2010).

The differences of availability of lowlands versus highland areas could be related to the observed differences of orographic patterns of speciation among regions. For example, speciation events involving lowlands were more frequent in South America where Amazonia, Cerrado, Caatinga and Choco occupy vast land extensions and tropical Andes and Atlantic mountains are most restricted (Eva et al. 2002), whereas speciation between and within mountain ranges was more common in Middle America where orography is more complex (Marshall 2007). Hot spots of pitviper speciation are especially prominent in the Mexican and Middle American mountains (Castoe et al. 2009, Bryson et al. 2011, Mason et al. 2018) where allopatric species pairs of *Crotalus*, *Metlapilcotalus*, *Bothriechis* and *Cerrophidion* arose separated by intervening lowlands.

Species richness was higher at lower elevations, maximum at medium, decreasing posteriorly with the altitude, thus following a similar pattern that found in other ectotherms like frogs or salamanders (Hutter et al. 2007, Smith et al. 2007, Wiens et al. 2007) and found in some reptiles (McCain 2010).

The decrease of species richness with elevation could be caused by environmental constraints such as the lower temperatures experienced (McCain 2010, Jins et al. 2021), low productivity or less diversity of habitats available in the mountain tops. Conversely, resource availability might provide more opportunities for ecological species segregation at lower elevations. As a result, species living at low altitudes might have more varied niches available, thus decreasing competition among species and consequently reducing extinction, which ultimately can drive high species richness, habitat diversity and wide range of body sizes (Jadin et al. 2019). However, in comparison with lowlands, medium altitudes hold the maximum species richness in New World pitvipers. Because the ancestral pitviper was inferred to inhabit this elevational zone, lineages originated at medium elevation could have had more time for speciation (montane museum hypothesis, Smith et al. 2007). Nevertheless, I found that the time of colonization of the bands was not significantly associated with its species richness. Thus, it is possible that ancestral pitvipers living at medium elevations experienced high speciation rates (montane species pump hypothesis, Smith et al. 2007). In accordance with this explanation, the analysis of diversification and speciation indicated fast rates at the base of the phylogeny where the initial ancestral lineages were reconstructed to live at medium elevation and the best QuaSEE model accounts for the hump-shaped response of speciation on elevational variation. In contrast, the negative association of the DR metric and the negative correlation of the ES-sim test with altitude indicates that the accumulation of speciation events though time was highest in lowland species. The posterior slowing of the rates is congruent with other studies (Alencar et al. 2016) and suggests a saturation effect on the species communities when after initial speciation in medium elevations vipers colonized the lowest and highest, occupying the available niches. Thus, after initial high speciation rates accumulated by lineages inhabiting medium elevations, they experienced an asymmetric decline inversely correlated with elevation when other altitudinal zones were colonized. This process might explain an elevational pattern of species richness biased towards medium elevations while keeping high values in lowlands and few species at high altitudes. Nevertheless, the positive association between species richness and number of colonizations, which increase with the decrease in altitude when the finest band scheme was analysed, suggests that the role of the phylogenetic history cannot be completely ruled out.

In summary, since the colonization of the New World (Kraus et al. 1996) mountains have become drivers of the diversification of pitvipers by hosting more speciation events than lowlands, leading to high endemicity (Rodriguez et al. 2016) and generating the highest species richness at medium elevations through changes in speciation rates. Lowlands experience more dramatic anthropogenic alteration than mountains because human population is heavily concentrated in lower altitudes (Cohen & Small 1998), thus placing under most pressure the areas that hold high species richness and lowland endemics (Porras et al. 2013, Birskis-Barros et al. 2019). However, speciation by isolation in mountain ranges gives rise to many montane endemic pitvipers (e.g. *Cerrophidion*, Jadin et al. 2012; *Mixcoatlus*, Jadin et al. 2011) which have quantitative ecological and evolutionary distinctiveness and are amongst the most endangered vipers in the world, rivalling only insular endemics (Maritz et al. 2006). Mountain endemics are testimonials of the multiple colonization of mountains by pitvipers through the evolutionary history of the New World lineage, which leads to species with small ranges (Jadin 2010) whose conservation relies on the preservation of the pine-oak and tropical deciduous forests dramatically affected by human alteration of these habitats.

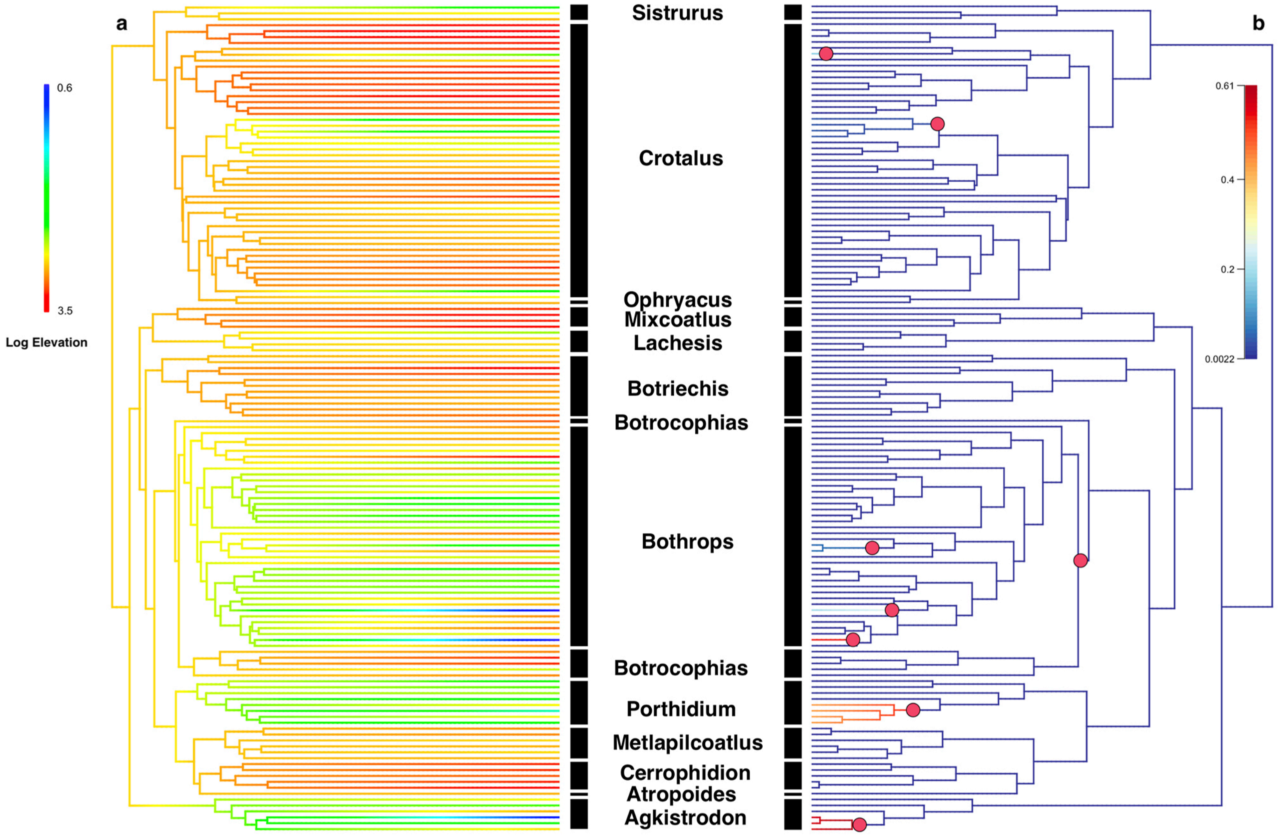

Figure 4. (a) Maximum likelihood reconstruction of the ancestral log-elevational mid-point of New World pitvipers based on the best fitted-model (Lambda). (b) Phylorate and best shift configuration representing the estimated rates of elevational evolution obtained by BAMM analysis. Branches of both analyses are coloured with the colours of the corresponding scales.

Note added in proof

After finishing the analysis of this research und manuscript preparation, an extensive revision of the taxonomy of *Bothriechis schlegelii* was published, leading to the recognition of eight additional species (Arteaga at al. 2024), which were not considered in this study. Maybe the exclusion of these results herein is tolerable, as the only well-supported sister relationship in the phylogeny of this paper is between *Bothriechis hussaini* and *B. nitidus*, two lowland species from southern and northern Ecuador. Moreover, there has already been some critical debate on the respective publication (Reyes-Velasco 2024).

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Supplementary data

The following data are available online:

Supplementary material 1. Accession numbers of the DNA sequences of the New World pitviper species included in the analysis.

Supplementary material 2. Biogeographic areas used in BioGeoBEARS analysis.

Supplementary material 3. Elevational ranges and mid-elevation points of New World pitviper species analysed in this study.

Supplementary material 4. Phylogenetic relationship of the overall species dataset used in this study.